Scientific Abstract

Myelosuppression remains a major dose limiting toxicity of many chemotherapeutic agents. In an effort to combat this, a number of gene therapy approaches have been initiated to overexpress drug resistance genes in hematopoietic progenitors [HPs] with the intent of reducing chemotherapy induced myelosuppression. One class of alkylating chemotherapeutic agents, the nitrosoureas, is associated with cumulative myelosuppression, persistent marrow damage and delayed secondary leukemias, most likely due to direct toxicity to early hematopoietic progenitors. This group of agents attack the O^6 position of guanine and exert their cytotoxic effect on this basis. We have shown that an important drug resistance gene for these agents, O^6-alkylguanine-DNA alkyltransferase [AGT], encoded by MGMT, repairs O^6-alkylguanine lesions and reduce its cytotoxicity . Many tumors express high levels of AGT and are resistant to this class of agents, whereas AGT is expressed at low levels in hematopoietic progenitors. This drug sensitivity pattern is inverse to that which would provide an optimal therapeutic index and may explain why myelosuppression occurs at doses which are unable to achieve effective tumor response.

Two recent observations make possible a gene transfer approach to test the hypothesis that overexpression of MGMT will protect the bone marrow from nitrosoureas and that transduced cells will have a selective survival advantage in vivo. First, the AGT inhibitor, O^6-benzylguanine, [BG], markedly sensitizes tumors to BCNU in xenograft models and, in our early clinical trials, is able to deplete AGT in patient tumor and normal cells. Second, a mutant MGMT, glycine 156 to alanine, [ΔMGMT] has been identified which is resistant to BG inactivation. In order to protect hematopoietic progenitors from BG & BCNU, the MFG based retroviral vector containing G156A mutant MGMT [ΔMGMT] was synthesized. ΔMGMT transduced human CFUs and LTCICs and murine bone marrow progenitors were found to be significantly resistant to BG & BCNU in vitro. Furthermore, mice transplanted with ΔMGMT transduced cells had a significant survival advantage over control animals when challenged by repetitive doses of BG & BCNU. These mice also showed enrichment of the transduced progenitors in vivo. On this basis, we propose that retroviral expression of ΔMGMT in human hematopoietic progenitors will reduce myelosuppression after therapy with BG & BCNU. As a consequence, therapy with BG & BCNU can be optimized by sensitizing the tumor with BG and making hematopoietic cells resistant to BG with ΔMGMT. This potential to reverse the sensitivity pattern of tumor vs. hematopoietic cells represents a uniquely different approach to drug resistance gene therapy.

We plan to conduct a clinical study consisting of 12 evaluable subjects to determine the feasibility and biologic relevance of this approach with the following objectives:

1 To evaluate the feasibility of introducing and expressing mutant-MGMT-G156A (ΔMGMT) cDNA in hematopoietic progenitors taken from advanced solid tumor patients using a safety modified retroviral vector MFG.

2 To determine the toxicity associated with reinfusion of *ex vivo* transduced hematopoietic cells into patients with advanced cancer, including the detection of replication competent retrovirus.

3 To evaluate the feasibility of identifying ΔMGMT transduced and BG & BCNU resistant hematopoietic cells from the bone marrow of patients infused with ΔMGMT transduced CD34 cells.

4 To evaluate the feasibility of *in vivo* enrichment of the transduced hematopoietic progenitors by repeated treatment of patients with BG & BCNU.

5 To evaluate the toxicity of repeated BG & BCNU treatments in patients who received ΔMGMT transduced CD34 cells.